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# BIOLOGICAL BULLETIN

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*Vol. XXXV.* *October, 1918.* *No. 4.*

# BIOLOGICAL BULLETIN

## TWO NEW EYE COLORS IN THE THIRD CHROMOSOME OF DROSOPHILA MELANOGASTER.

MILDRED HOGE RICHARDS,
CRAWFORDSVILLE, INDIANA.

In the cultures of *Drosophila melanogaster* which I have been breeding at Indiana University, two new eye colors have lately appeared. One of these, rose, closely resembles pink and peach of the third chromosome, and is allelomorphic to them. The other eye color, scarlet, is a bright red much like the vermilion of the first chromosome in appearance, but quite distinct from it genetically, for it is located on the third chromosome.

Both eye color mutations occurred in the winter of 1916. Scarlet appeared (November 18, 1916) in a wild stock which was being used in a temperature experiment. Rose appeared (January 9, 1917) after scarlet had been crossed to eyeless, and later was found in the eyeless stock, so that the mutation evidently had occurred there.

A series of temperature experiments was undertaken in the fall of 1916 in the effort to determine whether it would be possible to adjust a strain of *Drosophila* to a temperature abnormally high for that species. The flies were bred in pairs, the temperature of the incubator usually being maintained at 27–31°. Each generation of flies was raised, during the greater part of its period of development, at this higher temperature. The flies would not lay, however, under the abnormal conditions; they were allowed to mate, therefore, at room temperature, after which they were transferred to the incubator. The larvæ having pupated, the cultures were again removed from the incubator, for the heat proved fatal to the pupa stages. Even with these

precautions all of the flies died if the temperature was allowed to rise above 33°.

Although from the standpoint of its original purpose the experiment, due to inadequate apparatus, was not entirely successful, a number of interesting things resulted from it. Abnormalities of various kinds appeared in the second generation of incubated flies. In one case, after the first generation had been developed in the incubator, some of the progeny ($F_1$) were bred at room temperature, and among the $F_2$ so obtained arose the scarlet eye color.

The temperature experiments were carried on for seven generations; and in each variations appeared. Most of these were peculiarities of the wings, but there were also modifications of eye and leg characters. Among the wing peculiarities were the following: short wings, wing edges turned up, wings folded at the tip, wings bent at abnormal angles, wings spread similar to "spread" of the third chromosome, and wings of odd shapes. Still other flies had weak legs, bent between the joints, and one fly had small eyes.

In each case the variant was allowed to mate with a wild fly at room temperature. The $F_1$ were divided into two lots, one of which reproduced at room temperature and the other in the incubator. It was hoped in this way to perpetuate the variations, should any of them be germinal. Many of the flies were nonviable or non-fertile, but in those cases where $F_2$ were obtained they were usually normal, and the abnormals which did appear were not like those of the original parent.

Only one color variation appeared. It proved to be germinal in character, and has given rise to the strain called scarlet.

Scarlet is a bright color, like the vermilion of the first chromosome, in flies that are newly hatched. As the flies get older the color gradually changes, and in old flies it is almost indistinguishable from the wild red type. In all crosses where scarlet is used it is highly important, therefore, to count the flies immediately after hatching.

Scarlet was crossed with a member of each of the four groups of linked genes in order to determine its linkage. Crosses were made with blood of the first chromosome, with vestigial of the

second, with sooty of the third, and with eyeless of the fourth. The results of these crosses demonstrated that scarlet is a member of the third group of linked genes. When scarlet was crossed to blood, vestigial, or eyeless, all possible combinations were formed in $F_2$, showing that independent assortment occurs between these genes and scarlet. However, when scarlet was crossed to sooty, no scarlet sooty flies were obtained in $F_2$. The crosses gave the following results:

1. Scarlet ♂ × Blood ♀ (first chromosome character).

$F_1$ Wild-type ♀, blood ♂

$F_2$
- Wild-type ♂s and ♀s 178
- Blood ♂s and ♀s 156
- Scarlet ♂s and ♀s 70
- Blood scarlet ♂s and ♀s 50

2. Scarlet × Vestigial (second chromosome character).

$F_1$ Wild-type

$F_2$
- Wild-type 312
- Vestigial 93
- Scarlet 109
- Vestigial scarlet 38

This is evidently a 9 : 3 : 3 : 1 ratio.

3. Scarlet × Sooty (third chromosome character).

$F_1$ Wild-type

$F_2$
- Wild-type 611
- Scarlet 265
- Sooty 284
- Scarlet sooty 0

4. Scarlet × Eyeless (fourth chromosome character).

$F_1$ Wild-type

$F_2$
- Wild-type
- Scarlet
- Eyeless
- Scarlet eyeless

Among the $F_2$ of this cross appeared the new eye color which has given rise to the strain called rose. This new eye color was later found in the eyeless stock itself.

The exact linkage relations of scarlet were next determined by mating scarlet to sooty and to dichæte, and back crossing the

$F_1$. Genes for these two characters are located quite far apart in the third chromosome.

Scarlet × sooty

$F_1$ Wild-type

$F_1$ ♀ back crossed to scarlet sooty ♂ gave the following:

$F_2$ non cross overs { Scarlet 1230; Sooty 1511 }

$F_2$ cross overs { Wild-type 695; Scarlet sooty 623 }

Percentage of crossing over was 32.4

Dichæte × scarlet. Dichæte is a dominant character, non-viable in the homozygous condition. The dichæte to which scarlet was crossed was therefore heterozygous. The $F_1$ dichæte females were back crossed to scarlet males and the percentage of crossing over thus immediately obtained in the $F_2$.

Non cross over classes { Scarlet 781; Dichæte 952 }

Cross over classes { Scarlet dichæte 23; Wild-type 45 }

Percentage of crossing over is therefore 3.2.

Scarlet is thus placed at a locus of 3.2 from dichæte, and 32.4 from sooty.

According to unpublished work from the laboratory at Columbia University, the approximate loci for these third chromosome genes are the following:

Dichæte 11
Pink and peach 16
Sooty 34

Since such a large per cent. of cross over was obtained for scarlet sooty (32.4), scarlet is probably at the left of dichæte (on the sepia side), sepia being at 0. Since it is 3.2 from dichæte, it is at the approximate locus of 7.8, (11 − 3.2).

Since dichæte is 23 to the left of sooty, (34 − 11), we should expect the value of scarlet sooty to be 3.2 + 23 or 26.2, instead of 32.4. The larger value obtained in this work may have been due of one of several causes. Genetic factors which influence the amount of crossing over have been found by Sturtevant and Muller for the third chromosome group. Plough has also

shown that in some cases external conditions (temperature) change the amount of crossing over.

Since the appearance of scarlet, the same mutation has been found at Columbia University by D. E. Lancefield. Lancefield's paper will be found in this journal. The two strains arose quite independently, in laboratories remote from each other, and in stocks that were absolutely distinct. They are identical in appearance and in genetic constitution, and are due to the mutation of the same gene, but the process of mutation occurred at different times and in different localities. That they are due to mutation of the same gene is demonstrated by crossing the two stocks together, as I have done, using the stock kindly sent me from Columbia and my own strain. The $F_1$ flies from this cross were exactly like the parents. This origin of the same mutation in two widely separated laboratories is of particular interest, for here there is no possibility of contamination.

The mutation rose occurred in the eyeless stock, but was not observed until eyeless was crossed with scarlet. In color rose, which is somewhat lighter than pink, is a shade lighter than peach in older flies, but is practically identical with the latter at the time of hatching. When rose was first observed there was no peach stock kept in the laboratory. The three colors are very similar when the flies are first hatched, but rose does not become as dark with age as do the others.

At the time when rose appeared, Dr. F. Payne had running in the same laboratory stock of a new eye color identical in appearance with rose. At that time it was thought that rose had been derived from this stock by contamination. The gene for Dr. Payne's eye color, called salmon, and as yet unreported, had already been located in the sex chromosome. When salmon was crossed with rose, the following results were obtained.

Cross 1. { Salmon ♂ × rose ♀
$F_1$ wild-type.

Cross 2. { Rose ♂ × salmon ♀
$F_1$ Salmon ♂ and wild-type ♀

These results indicated at once that the two eye colors are not the same genetically, since rose is not sex linked. The $F_1$ when inbred produced only the two eye colors, wild-type and salmon

(or rose). Since rose and salmon are indistinguishable phenotypes, the results correspond closely with the expectation as will be seen in the following table. In these results the classes rose, salmon, and the double recessive rose salmon are all alike in appearance and therefore could not be separated.

| Classes Expected.[1] | | Percentage Expected. | | Percentage Obtained. | Actual Numbers Obtained. |
|---|---|---|---|---|---|
| *Cross 1.* | | | | | |
| Red ♀ | | | 37.5 | 37.3 | 590 |
| Rose ♀ | | | 12.5 | 14.4 | 228 |
| Red ♂ | | | 18.7 | 18.4 | 291 |
| Rose | Salmon ♂ | 18.7 | | | |
| | Rose ♂ | 6.3 | 31.3 | 29.9 | 474 |
| | Salmon rose ♂ | 6.3 | | | |
| *Cross 2.* | | | | | |
| Red ♀ | | | 18.8 | 20.9 | 322 |
| Rose | Rose ♀ | 6.2 | | | |
| | Salmon ♀ | 18.8 | 31.2 | 31.4 | 483 |
| | Salmon rose ♀ | 6.2 | | | |
| Red ♂ | | | 18.8 | 17.7 | 273 |
| Rose | Rose ♂ | 6.2 | | | |
| | Salmon ♂ | 18.8 | 31.2 | 30.0 | 462 |
| | Salmon rose ♂ | 6.2 | | | |

This is another case similar to the well known one of the white sweet peas, in which two different genes produce the same phenotype.

Since rose was not linked to the first chromosome group, it was crossed to vestigial of the second, and to sooty of the third group. The first cross gave a 9 : 3 : 3 : 1 ratio, and the second a 2 : 1 : 1 ratio, as follows:

Rose × vestigial

$F_1$ Wild-type

$F_2$ Wild-type 1046, vestigial 257, rose 345, vestigial rose 104.

Rose × sooty

$F_1$ Wild-type

$F_2$ Wild-type 727, rose 278, sooty 330, rose sooty 0.

These results show that there is free interchange between the genes for rose and for vestigial, but that rose and sooty are in the same chromosome pair. Rose is therefore a member of the third chromosome group. Its linkage relations were determined

[1] Expectation is calculated by the checkerboard method, on the basis of 16 $F_2$ individuals.

by mating the $F_1$ female from the cross sooty × rose, to a rose sooty male fly. The $F_1$ female was used instead of the $F_1$ male for the reason that there is no crossing over in the male of this species. The double recessive rose sooty had been obtained by mating $F_2$ sooty by $F_2$ rose flies. The percentage of cross over from the back cross was 21.1. This therefore is the strength of the linkage between sooty and rose.

$F_2$ Non cross overs { Rose 647 / Sooty 634
$F_2$ Cross overs { Rose sooty 173 / Wild-type 170

Percentage of cross over, 21.1.

Rose was next crossed to pink, a third chromosome character of known linkage and with locus 16. The $F_1$ were intermediate between the two parents, and when inbred gave only $F_2$ of the same eye colors. This showed conclusively that pink and rose are allelomorphic.

The linkage relations of rose should of necessity be the same as those of pink. According to previous work pink had been placed at a distance of about 18 units from sooty, whereas the present data indicate a distance of 21.1 units between these factors. This small difference in the percentage of crossing over may be due to one of a number of causes.

Another allelomorph of pink, peach, had previously been identified. When peach is crossed to rose, flies with intermediate eyes resulted. Rose is therefore the third of a series of allelomorphs at the approximate locus of 16 on the third chromosome.

## Summary.

In a series of temperature experiments, during which cultures of *Drosophila melanogaster* were incubated, many new variations appeared. Only one, scarlet eye color, similar in appearance to "vermilion" of the first chromosome group, was found to be a true mutation. Scarlet is a third chromosome character whose gene is on the sepia side of dichæte at a locus of 3.2 from the latter. The same eye color has arisen as an independent mutation at Columbia University in the cultures of D. E. Lancefield, whose paper is also in this journal. When crossed, the two strains give $F_1$ like the parents.

Rose, an eye color appearing in another stock, is the third of a series of allelomorphs. It occupies the same locus on the third chromosome as do pink and peach. Rose is identical in appearance with salmon, a new sex linked mutant found by Dr. F. Payne. This case furnishes another example of different genes producing the same phenotype.

June, 1918.

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# SCARLET, AN AUTOSOMAL EYE COLOR IDENTICAL WITH SEX-LINKED VERMILION.

D. E. LANCEFIELD,

COLUMBIA UNIVERSITY.

Vermilion, a sex-linked eye color in *Drosophila melanogaster* (*ampelophila*), is duplicated in appearance by a new mutant called scarlet (symbol $s_t$). The gene for scarlet is located in the third chromosome to the left of dichæte. In spite of this difference of location of the genes for the two eye colors they are quite indistinguishable in appearance and can not be separated from each other when mixed. Here we have a case in *Drosophila* like the well-known case of the two whites in sweet peas.

Upon crossing scarlet and vermilion, wild-type red is produced. Reciprocal crosses between scarlet and vermilion cut-winged flies from stock gave the following results in $F_1$. A vermilion cut male mated to a scarlet female produced offspring entirely wild-type in appearance both for eyes and wings. Reciprocally a scarlet male by a vermilion cut female gave wild-type daughters, while the sons showed the sex-linked characteristics of the mother, viz., vermilion eyes and cut wings.

The next generation was not obtained because the writer entered military service before completing it, but the significant fact is demonstrated already that in *Drosophila* as elsewhere, indistinguishable characters may be produced by differently located genes.

## ORIGIN OF SCARLET AND PINKOID.

The origin of scarlet was rather remarkable as it first appeared in a strain which already contained eosin, and at the same time was found a new eye color, pinkoid, whose gene is in the second chromosome. In a cross of a bar male to an eosin miniature female of non-disjunctional stock, there appeared in the offspring 38 females which were normal in appearance and did *not* have the heterozygous bar eye. These females were due to reversion

of the bar gene. One of these females, which was heterozygous for eosin and miniature, was crossed to an eosin miniature brother. The offspring showed no bar, but the eosin flies were very variable, some appearing darker than usual and some lighter like eosin. This was probably totally unrelated to the reversion of the bar factor, for the next generation, produced from eosin flies of the above mating, showed unexpected results. A great variety of eye colors were present. Two of these were the two new mutants found March 18, 1918 and the other colors were different combinations with eosin. The scarlet individuals were thought to be vermilion until the gene was found to be not sex-linked. The pinkoid flies had an eye-color very closely resembling that of the mutant race known as pink, but had the further interesting characteristic that the wings were also very greatly affected, resembling typically the wings of the "inflated" stock, which is sex-linked. When the eyes were pinkoid the wings were always abnormal, being either inflated as if by a bubble of air or else being shortened and wrinkled. That both the eye-color and the wing character are the result of the same gene is probable because they appeared at the same time in the same individuals and all efforts to obtain the eye-color separate by outcrossing and back crossing have failed.

Owing to the circumstances surrounding the appearance of these two mutants, nothing can be said concerning the stock from which they arose. When first observed, eosin was also present and altogether a most remarkable array of colors was produced. The homozygous form of either new mutant with eosin is white in appearance. The colors ranged from this multiple white up through cream, lemon, eosin, orange, pinkoid to normal red; according to the combination existing in any one individual.

## Location of New Genes.

Pinkoid and scarlet were not sex-linked and were found to be freely segregating from each other. Such segregations indicated that they were in separate autosomes. Accordingly they were each crossed to the double dominant form star dichæte, the genes of which are in the second and third chromosomes respectively. A star dichæte male resulting from these crosses was

then back crossed either to pinkoid or scarlet stock, as the case might be. Males were used thus because there is no crossing over in males in *melanogaster*. Pinkoid was found to be a second chromosome gene as no star fly was pinkoid but all not-star individuals were pinkoid without regard to whether or not they were also dichæte. The pinkoid strain had a very low viability, however, and was hard to keep going, so that it is not a satisfactory character for use in genetic experiments. The strain died out while efforts were being made to place the gene in the second chromosome by its linkage to other second chromosome genes.

By a similar cross, scarlet was found to be in the third chromosome; as a star dichæte male, heterozygous for scarlet, back crossed to a scarlet female produced dichæte flies which were never scarlet, and not-dichæte flies which were always scarlet.

Further tests were made to dichæte and to hairless in the third chromosome by crossing scarlet to each and back crossing the heterozygous hairless or dichæte females to pure scarlet males. The results indicated that scarlet is to the left of dichæte in the chromosome, though more extensive data should be obtained. The counts from three cultures gave 534 non-crossovers and 15 crossovers between dichæte and scarlet, or a percentage of crossing-over of 2.7. Three cultures to test linkage to hairless produced a total of 552 flies of which 140 were in crossover classes, which gives a crossover value of 25.4 per cent. According to unpublished data of Bridges and Gowen the crossover percentage of dichæte and hairless is 18.6. The data here recorded indicate that scarlet is to the left of dichæte, as scarlet gave a crossover value of 25.4 from hairless and scarlet is not far enough from dichæte to be to the right of hairless. However, the relative order of scarlet and dichæte can be definitely settled by more data and by using scarlet with two loci of known position simultaneously.

## Summary.

1. Scarlet is a new eye color identical in appearance with vermilion but in the third chromosome.

2. When scarlet is crossed to vermilion, normal red eyes result, except where sons inherit vermilion from their mother.

3. Scarlet gives a crossover percentage of 2.7 with dichæte and 25.4 with hairless.

4. Pinkoid is a new second chromosome mutant that appeared contemporaneously with scarlet, and that affects both the color of the eye and the shape of the wings.

COLUMBIA UNIVERSITY,
May, 1918.

# THE TOXIC ACTION OF KCN AND ITS RELATION TO THE STATE OF NUTRITION AND AGE OF THE CELL AS SHOWN BY PARAMECIUM AND DIDINIUM.[1]

BARBARA LEE LUND.

## INTRODUCTION.

Many results from studies on behavior and inheritance in the unicellular organisms show that the same cell is different at different times. The precise conditions under which these differences arise have been only incompletely determined, and in relatively few instances do we know enough about the conditions of their occurrence to be able to repeat exactly the results. In short, physiology knows that differences of this or that kind occur among cells of the same cell species (*i. e.*, cells which are apparently identical in outward appearances and history), but knows very little about how they occur and what the changes are in the cell that cause *difference in response to identical external conditions at different times.*

One of the striking instances of this variability is commonly observed when individuals from a pure line of *Paramecium* or other protozoa are taken from the same culture and placed in solutions of various kinds of chemicals. Some of the cells die quickly, others survive for long periods of time. What are the origin and causes of such differences among individuals of a pure line population? The following is an attempt to solve some of the features of this problem.[2] In order to increase the general significance of the results the experiments have been carried out on *Didinium nasutum* as well as on *Paramecium caudatum.* Preliminary experiments on *Paramecium* indicated that the state of nutrition played an important rôle in determining the results, so that *Didinium* was selected because its food (*Paramecium*)

[1] From the Department of Animal Biology of the University of Minnesota.

[2] For discussion of certain phases of this problem, see Jennings, "Behavior of the Lower Organisms," Chap. XVI.

can be controlled both as to quality (a pure line of *Paramecium*) and as to quantity if necessary. Later it was discovered that an equally good control of the food of *Paramecium* could be obtained by feeding yeast. A special study of the possibilities and limits of the use of pure yeast as a food for *Paramecium* is in progress.

The problem of the cause of the differences in resistance to the toxic action of potassium cyanide which *Paramecium* and *Didinium* individuals show was chosen because it has very generally been assumed that the toxic action of the cyanides on other organisms is specifically due to their ability to inhibit intracellular oxidations, and that differences in survival time in cyanide solutions were directly due to the difference in the rate of oxidations; or, as more generally expressed, "rate of metabolism" in the organisms studied (Child, '15). Does difference in resistance of *Paramecia* or *Didinia* to KCN depend upon the rate of oxidations in the cell?

The variety of types of cell suitable for studies on cell individuals under normal conditions is very limited. Most of the information along this line has been obtained from blood cells, yeast cells, echinoderm eggs and protozoa. The most extensive and accurate physiological data on such questions as the nature of the toxic action of the cyanides, rate of intracellular oxidations and its changes in the cell during the period of cell division and during the time between successive cell divisions, etc., have been obtained from experiments on echinoderm eggs. It is interesting therefore to see to what extent a comparison can be made of the results on echinoderm eggs with those on unicellular organisms during division and the changes which occur in a protozoan cell between two successive divisions. For this reason I have given below a brief review of the literature on the changes that occur in the echinoderm egg during cleavage.

It is important to note that the following results on survival time in the solutions of KCN were obtained not by observing large numbers of cells at a time, as is usual in this type of experiment, but instead by observing one or two individuals at a time and recording the survival time of each individual, so that the averages given are averages of the survival times of indi-

vidual cells with a definitely known history. This necessitated a great deal more labor, but was necessary in order to know exactly the age and history of each cell that was used.

## EXPERIMENTAL.

The first two experiments were preliminary and served to orient the problem.

*Experiment I.*—Suspecting that starvation of *Paramecia* had some relation to its power of survival in KCN solutions, a quantity of *Paramecia* from a pure line hay infusion culture was centrifuged and placed in one half native medium and one half tap water. This was used as a control. Twenty c.c. of this was added to a jar containing 600 c.c. tap water, on March 18; and the same to another jar, on March 19. I made no counts, but there were not over seven or eight hundred animals in each jar, so that there was a large volume for each individual. Forty-three of the control animals were tested immediately with the KCN. About twenty of those added to the pure tap water were tested as soon as added, and about the same number on each successive day following. The animal to be tested was transferred from the tap water to about 2 c.c. *N*/50 KCN solution in a watch glass by means of a capillary pipette. As small an amount of water as possible was transferred with the animal. It was then watched, a binocular microscope being used, until all movement ceased, cessation of movement being considered the death point. Soon after this, as a rule, the animal completely disintegrated. The average time of survival is given in Table I.

As can be seen by glancing at the table the survival time varies greatly in both lots, and apparently chaotically. The standard deviation[1] each day was large, varying from $0.63 \pm .06$, lot I., for the sixth day to $2.73 \pm .24$ on the fourth day; and similarly for lot II. This did not decrease as time went on.

Before proceeding further, the question arose as to whether the time of beginning cytolysis might be a more unvarying death point than the time of cessation of movement. If the animals are in the same condition as regards food and age then the death

[1] The standard deviation is a measure of the degree of variation. See C. B. Davenport, "Handbook of Statistical Methods."

## TABLE I.

Several hundred paramecia were transferred from a pure line mass culture to each of two jars of tap water, and about twenty of these animals were taken at random each day and treated with KCN. The averages of these survival times is given in the vertical column headed "Average survival time in minutes." The average of the survival times of 43 control individuals from the hay infusion was 2.19 minutes.

| | Lot I, March 18. | | | Lot II, March 19. | | |
|---|---|---|---|---|---|---|
| | Number Tested. | Average Survival Time in Minutes. | Standard Deviation. | Number Tested. | Average Survival Time in Minutes. | Standard Deviation. |
| 1st day..... | 23 | 3.289 | 1.11±.11 | 21 | 1.995 | 0.76±.07 |
| 2d day..... | 25 | 1.100 | 0.72±.06 | 22 | 0.870 | 0.64±.06 |
| 3d day..... | 25 | 1.132 | 1.09±.10 | 24 | 1.595 | 1.25±.12 |
| 4th day..... | 28 | 1.083 | 2.73±.24 | 20 | 0.717 | 1.54±.16 |
| 5th day..... | 22 | 3.190 | 0.94±.09 | 20 | 1.877 | 1.15±.12 |
| 6th day..... | 22 | 1.675 | 0.63±.06 | 21 | 0.954 | 0.65±.06 |
| 7th day..... | 22 | 1.279 | | 20 | 0.902 | |
| 8th day..... | 21 | 0.966 | | | | |
| 9th day..... | 19 | 1.157 | | | | |
| 10th day..... | 20 | 1.257 | | | | |

point which is the most constant should be used. I obtained dividing animals in the usual way (described below) and used the daughter cells as soon as division had taken place. Thus the animals were all the same age. The two sisters were placed in a watch crystal with 1 c.c. $N/75$ KCN solution, and the time of cessation of movement recorded. They were further watched and the time of beginning cytolysis recorded. Two experiments were carried out, 30 animals being tested in the first and 60 in the second. In order to obviate giving all the detailed data the standard deviation, which is a measure of the degree of variation, is given. In the first experiment the standard deviation for the cessation of movement was found to be $2.74 \pm .23$, while for cytolysis it was $3.86 \pm .33$. In the second the figures were $1.08 \pm .06$ for cessation of movement, and $2.37 \pm .13$ for cytolysis. Thus it is seen that the time of cessation of movement in KCN is less variable, and throughout this work I have used it as the "death point."

*Experiment II.*—Since the animals in experiment I. were taken at random from a pure line mass culture it is apparent that (1) some of them must have been old and some of them young;

(2) some of them had also probably eaten more recently than others, containing therefore more food in vacuoles. Could these two factors account for the random variation from day to day, and the deviation in each day's experiment?

To answer this question I first attempted to see whether there was any difference in the survival time between old and young *Paramecia* when they were treated with *N*/85 KCN solution. A pure line of *Paramecia* was cultivated in Syracuse watch glasses in hay infusion. About eight fresh watch glasses were set and seeded with the same pure line every day so that a constant supply of dividing animals was at hand. The experiment consisted of four parts:

*Part I.:* The watch glass cultures were looked over and animals with constrictions isolated in watch crystals. As soon as one divided one of the sisters was picked out with a capillary pipette, washed in one c.c. tap water for about thirty seconds, and then placed in *N*/85 KCN solution and the death point noted; while the other sister was left to age in tap water. Thirty pairs were so treated, the older sisters being killed at invervals varying from 25 to 215 minutes. The average survival time of the older sisters was less than that of the younger sisters.

*Part II.:* Thinking that the tap water might have something to do with this result I repeated the experiment but eliminated tap water; that is, I diluted the KCN with distilled water and allowed the sisters to age in native medium (hay infusion). The average survival time of the older sisters was now greater than that of the younger.

*Part III.:* Is this difference in result observed in parts I. and II. due to the fact that the KCN was diluted with tap water in one case and with distilled water in the other, or to the fact that the animals aged in tap water in one case and in native hay infusion in the other? To determine this I repeated the experiment using KCN diluted with distilled water, and allowed the animals to age in tap water. The average survival time of the older sisters was again less than that of the younger, as in part I.

*Part IV.:* I repeated the experiment, using KCN diluted with tap water, and allowed the animals to age in native hay infusion. The average survival time of the older sisters was greater than that of the younger. These results are summarized in Table II.

*Conclusion:* The older animals are evidently more resistant to KCN than the younger, and the apparent exceptions shown in parts I. and III. are due to the fact that the animals were left to age in tap water instead of in hay infusion. This was probably due to inanition and not to any deleterious effect of the tap water itself. See experiment VI. below.

TABLE II.

SUMMARY OF RESULTS OF EXPERIMENT II., PARTS I., II., III. AND IV.

| | I (30 Pairs). | II (30 Pairs). | III (30 Pairs). | IV (30 Pairs). |
|---|---|---|---|---|
| | KCN Diluted with Tap Water. Animals Left to Age in Tap Water. | KCN Diluted with Distilled Water. Animals Left to Age in Native Hay Infusion. | KCN Diluted with Distilled Water. Animals Left to Age in Tap Water. | KCN Diluted with Tap Water. Animals Left to Age in Native Hay Infusion. |
| Average survival time of older sisters compared to younger..... | less | greater | less | greater |

*Experiment III.*—In performing the preceding experiments the question arose as to whether the effects of ageing in native medium were cumulative; that is, whether the resistance increased regularly as age advanced. To answer this question the following experiment was performed. The procedure was the same as in experiment II. except that $N/75$ KCN was used. One sister was killed in this immediately after division. The other sister was allowed to grow in the native medium (hay infusion) and killed at ages varying from 10 to 600 minutes. Thirty *Paramecia* were tested in each case; that is, thirty "young" and thirty "old" for each time interval. Averages of the survival times for the young and the old for each time interval were made, and in every case the *average* length of life after treatment with KCN was greater in the case of the old than of the young animals.

The results of this experiment are summarized by giving these averages and the difference between them (Table III.). They are plotted in curves (Fig. 1).

The difference between the averages increases as age of the surviving sister increases. The degree of variation in survival time is relatively small when compared to the average survival

time, showing that the results are comparable and real; that is, that there are actual differences in survival time. This indicates so far as this experiment goes that there is an *absolute increase* in the survival time of the cells in hay infusion from one division to the next division, rather than that the increase is due to decrease in survival time of their sisters killed immediately after division; for the survival time of the latter tended to remain about the same throughout the experiment as shown by the horizontal trend of curve *C*.

## TABLE III.

*Paramecium.*

*A*. Time in minutes between division of *Paramecium* and the killing of one sister in *N*/75 KCN.

*B* and *C* are self explanatory, each number representing an average of thirty individuals.

*D*. Average difference in survival time between the paramecia which were killed immediately after dividing and their sisters which were killed at the above intervals after living in native hay infusion. Each number represents the difference between the two preceding numbers in the same column.

| Age of "old" in mins. | *A* | 10 | 20 | 30 | 40 | 60 | 120 | 180 | 300 | 480 | 600 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Average survival time in minutes of "old" sisters | *B* | 2.22 | 2.62 | 3.30 | 3.35 | 2.85 | 2.90 | 3.00 | 3.11 | 3.55 | 3.71 |
| Average survival time in minutes of "young" sisters | *C* | 2.09 | 2.19 | 2.90 | 2.74 | 2.30 | 2.16 | 2.07 | 2.09 | 2.24 | 2.55 |
| Difference | *D* | .13 | .43 | .40 | .61 | .55 | .74 | .93 | 1.02 | 1.31 | 1.16 |
| Standard deviation of "old" sisters | *F* | 0.51 ±.04 | 0.34 ±.03 | 0.36 ±.03 | 0.72 ±.06 | 0.64 ±.06 | 0.47 ±.05 | 0.48 ±.05 | 0.40 ±.04 | 0.85 ±.07 | 0.67 ±.07 |

We may then briefly summarize the results from the previous experiments on *Paramecium* by saying that these have clearly shown that beginning at division there is an increase in the resistance of *Paramecium* to KCN when it is permitted to live in hay infusion, while if placed in tap water the resistance decreases.

By using hay infusion it would be practically impossible to show in *Paramecium* that these results are due to the state of

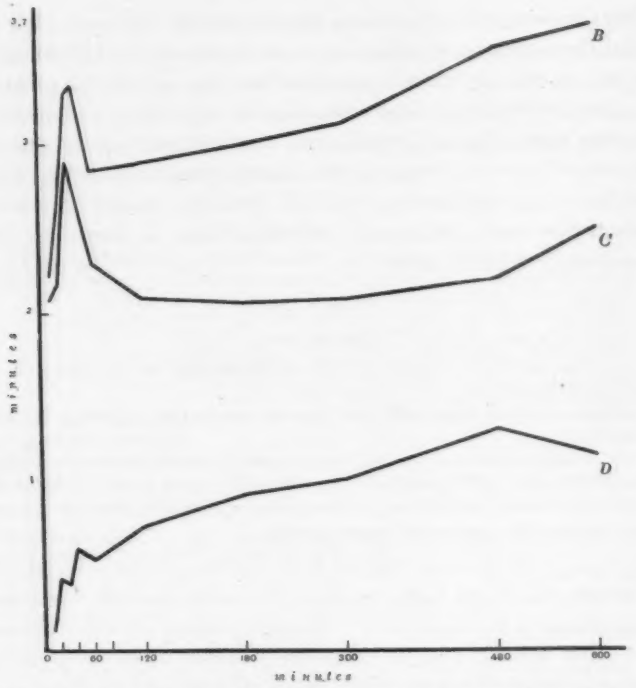


FIG. 1. *Paramecium.* Survival time of "old" and "young" paramecia in KCN. (See Table III.) Curve *B* represents the average survival time of "old" sisters. Curve *C* represents the average survival time of the "young" sisters of *B*. Curve *D* represents the difference between the average survival times of the "old" and "young" sisters. That is, it is the difference between the curves *B* and *C*. The relatively long survival time at 30 and 40 minutes, curves *A* and *B*, is due to lower temperature.

nutrition of the cell; for if we wished to show that it was due to the bacteria serving as food it would be necessary to provide the same medium (say, tap water) for both sisters, while only one sister was fed with bacteria. To control the bacterial food of *Paramecia* is very difficult, hence *Didinia* were used in the following experiments. A pure line of *Paramecia* served as food for the *Didinia*, and since the *Paramecia* could be washed free from hay infusion and placed in tap water it was possible to have all experimental conditions for the two sister cells of *Didinia* exactly

the same, except presence or absence of food. This then supplied ideal conditions for answering the question as to whether the state of nutrition of the cell was the condition which determined the survival time of the cell in KCN.

*Experiment IV.—Didinia* were cultivated in tap water in Syracuse watch glasses and fed on freshly washed *Paramecia* taken from a pure line mass culture. Animals with constrictions were isolated in watch crystals in tap water, and immediately on separation one sister was transferred to another watch glass in which was about one c.c. tap water containing many *Paramecia*. The two dishes, one containing one of the pair in tap water, the other containing the other sister in tap water and *Paramecia*, were then placed side by side in a moist chamber, and later the two sisters were killed at the same time in the same one c.c. of $N/20$ KCN solution. A more concentrated solution of KCN was used here for convenience, to shorten the survival time, for *Didinium* is much more resistant to KCN than *Paramecium*. Occasionally it was impossible to tell the difference between the two sisters at the end of the desired period, the one placed with *Paramecia* evidently not having eaten; and in those cases they were killed separately but as near the same time as possible. By working with two binoculars it was possible to make the treatment of the two with KCN practically simultaneous. Thirty pairs were used in each case, so that sixty individuals were killed at the end of each period of ½, 1, 2, 3, 4 and 5 hours after division. The results are summarized in Table IV., and curves *C*, *E* and *D*, Fig. 2.

The average difference in survival time (row *D*) increases until the fourth hour, and in this lot three fed ones out of thirty-three had divided; while in the next lot, that of five hours, 37.5 per cent. or 18 out of the 48 individuals isolated had divided and division of the remainder was apparently not far off. It was impossible to get a record of a six hour lot on account of division. Since the starved individuals did not divide at the same rate as the fed ones, it was possible to continue that part of the experiment beyond five hours. Table IV. *B*. It will be noted that the average survival time of these starving individuals shows a marked decrease. This is shown in Fig. 2, curve *E*. Is the

## TABLE IV.

*Didinium.*

*A*. Time after division when the sisters were killed in *N*/20 KCN. One of them had been starving in tap water, the other feeding on Paramecia in tap water. The temperature for this experiment was between 23° and 24° C. throughout, with the exception of the 12-hour lot when the temperature went down to 19° C. This accounts for the longer survival time in that case.

*B* and *C* are self explanatory, each number representing an average of 30 individuals.

*D*. Difference between the two preceding figures in the same column. That is, each number represents the average difference between 30 starved and 30 fed Didinia sisters.

| Age of sisters in hours | *A* | 1/2 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 15 | 19 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average survival time of starved in minutes | *B* | 5.9 | 4.268 | 3.45 | 3.743 | 2.64 | 2.28 | 1.67 | 1.19 | 1.00 | 0.87 | 1.32 (?) | 0.805 | 0.72 | 0.50 |
| Average survival time of fed in minutes | *C* | 6.38 | 8.524 | 8.776 | 9.766 | 6.11 | 5.090 | | | | | | | | |
| Difference | *D* | 0.483 | 4.256 | 5.326 | 6.023 | 3.464 | 2.804 | | | | | | | | |

increasing difference (Fig. 2, curve *D*) up to the fourth hour due entirely to the fact that one sister has eaten while the other has not; or do the sisters, presumably identical at the time of separation, gradually diverge as a result of differing rates of maturity, apart from effects of nutrition, so that even though subjected to the same conditions they would show different degrees of resistance as time went on?

*Experiment V.*—To answer this question an experiment was carried out in the usual way, the dividing animals being isolated and killed with KCN. I found, however, that the concentration of KCN I had previously used did not bring out the small differences in survival time as well in this case where the sisters were in the same medium and both were without food, as a weaker solution. Thirty pairs or sixty individuals were killed at the end of each period of ½, 1, 2, 3, 4, 5, 6, 7, 8, 9, 12, 19 and 24 hours after division. Since there was no difference in appearance or in previous treatment of the sisters I arbitrarily placed the survival times of the animals which died first in one column, and that of their more resistant sisters in another, took an average of the thirty individuals in each case, and have given in Table V.

the average survival times of each and the differences between these averages. The difference is also plotted in Fig. 2, curve *A*.

These results demonstrate that in the same environment the differences in survival time of the sisters do not increase but

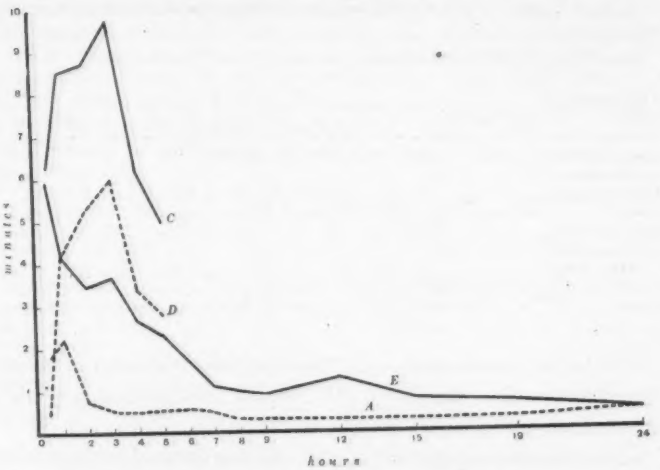


FIG. 2. *Didinium.* Survival time of Didinia in KCN. Summary of Tables IV. and V. The abscissa represents time in hours between division and death of Didinia. The ordinate represents survival time in minutes. Curve *C* represents the average survival time of fed Didinia. (See Table IV, *C*.) Curve *E* represents the average survival time of the starved sisters of *C*. (See Table IV., *E*.) Curve *D* represents the difference between the average survival times of fed and starved sisters. That is, it is the difference between *C* and *E*. (See Table IV., *D*.) Curve *A* represents the difference between the average survival times of the less and more resistant sisters, when both are starved. (See Table V., *A*.)

if anything decrease with time; *i. e.*, the cells tend to become more alike in their resistance to KCN, and therefore the large difference shown in experiment IV. must be due to the food one has eaten while the other has starved. Table V. shows that the difference in survival time at one half and one hour is greater than at any subsequent time. This is explained by the fact that division in *Didinium* is often unequal, one sister evidently containing more food in vacuoles than the other. As this food is used up by the cell the difference in survival time becomes less. (See experiment VI. below.)

## TABLE V.

*Didinium.*

*D.* Time after division at which the sisters, both of which had been subjected to the same treatment, were killed.

*B.* Each number represents the average survival time of 30 *Didinia.*

*C.* Each number represents the average survival time of the 30 sisters of the corresponding set of row *B.*

*A.* Differences between *B* and *C.*

| Age of sisters in hours..... | *D* | 1/2 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 19 | 24 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average survival time in minutes of less resistant sisters ..... | *B* | 3.57 | 3.30 | 2.93 | 2.49 | 2.60 | 2.18 | 1.45 | 1.53 | 1.80 | 1.59 | 1.69 | 2.22 | 2.51 |
| Average survival time in minutes of more resistant sisters.... | *C* | 5.37 | 5.50 | 3.61 | 3.02 | 3.12 | 2.69 | 2.04 | 1.97 | 2.12 | 2.42 | 2.02 | 2.56 | 3.10 |
| Difference..... | *A* | 1.80 | 2.20 | 0.68 | 0.53 | 0.52 | 0.51 | 0.59 | 0.44 | 0.32 | 0.83 | 0.33 | 0.34 | 0.59 |

Two other experiments on *Didinium* were carried out which were duplicates of experiment IV., except that saponin was used in one case and phenylurethane in the other, in place of KCN. The survival time varied with individuals apparently irrespective of the state of nutrition of the cell. The death points were less definite than in the case of KCN, and hence further efforts to trace the causes of difference in survival time in these substances was abandoned for the time being.

Some time after the preceding results were obtained it was found possible to grow pure lines of *Paramecia* in tap water by feeding them yeast, thereby making it possible to duplicate experiments IV. and V. on *Didinium.*

*Experiment VI.*—A pure line of *Paramecia* was raised in a suspension of fresh yeast and tap water. The *Paramecia* were centrifuged and washed thoroughly every morning, and added to a new suspension of fresh yeast to continue the culture. The cultures were kept in Syracuse watch glasses. If a culture is neglected and bacteria develop to any extent, the *Paramecia* become abnormal and finally die. If a starved *Paramecium* is put into a freshly made yeast suspension it eats greedily, and in a few hours increases greatly in size. From such yeast fed material, animals with constrictions were picked out with a

## TABLE VI.

*Paramecia*, YEAST-FED AND STARVED.

*A*. Time between division and killing the two sisters, one of which had lived in tap water, the other in tap water and fresh yeast suspension.

*B*. The averages of the survival times of 30 fed *Paramecia*.

*C*. Since all those left with food for 12 hours divided, the experiment was continued without food for 15-, 19- and 25-hour intervals. So that the numbers for 15, 19 and 25 hours, rows *C* and *D* represent average survival time of 30 starved sisters each.

*D*. The average survival time of the starved sisters of *B* from 0 to 12 hours and of *C* from 12 to and including 25 hours.

*E*. Differences between average survival time given in rows *B* and *D*, and between *C* and *D*.

| Age of sisters in hours ..... | *A* | 0 | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 12 | 15 | 19 | 25 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Average survival time of fed.... | *B* | 2.42 | 2.64 | 2.58 | 2.52 | 2.51 | 2.50 | 2.44 | 2.58 | 2.43 | 2.75 | all divided | | | |
| Average survival time of starved sisters of *D* ... | *C* | | | | | | | | | | | | 1.13 | 1.17 | 1.00 |
| Average survival time of starved | *D* | 2.40 | 2.24 | 1.88 | 1.80 | 1.84 | 1.79 | 1.74 | 1.77 | 1.56 | 1.78 | 1.59 | 1.11 | 1.14 | 1.05 |
| Difference..... | *E* | .02 | .04 | .70 | .72 | .67 | .76 | .70 | .81 | .87 | .97 | | .02 | .03 | .02 |

capillary pipette and placed in tap water. As soon as they separated one was placed in a watch glass of fresh yeast suspension, while the other was left in tap water. Sets of thirty each of such pairs were killed in *N*/75 KCN at intervals of 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours. It was impossible to continue this experiment longer than nine hours, for a large percentage of those which were fed, divided between the ninth and tenth hour. Sets of starved animals were continued however for 25 hours, being killed at intervals of 12, 15, 19 and 25 hours. The results are summarized in Table VI., and in the curves of Fig. 3.

The difference in survival time shows a general increase up to and including the ninth hour, the animals which were placed in the yeast supsension living longer than their sisters which were placed in clear tap water. The survival time of the latter showed a marked decrease with age. The experiment was continued, but instead of feeding one sister and starving the other, both were starved, as was done with *Didinia* in experiment V. above. Thirty pairs were left to starve in tap water for each period of 15, 19 and 25 hours, and then tested with KCN. I arbitrarily

placed the survival times of the sisters which died first in one column, and the more resistant sisters in another (though as a rule the survival times of sisters was the same), took an average of the two sets of sister cells of thirty individuals for each period and then found the differences between these averages, which I called the average difference between starved sisters, given in Table VI. *E*. This difference was very small, being .02, .03, and .02 minutes respectively for 15, 19 and 25 hours. This is practically the same as saying that there is no difference, since these numbers are within the limits of error in determining the

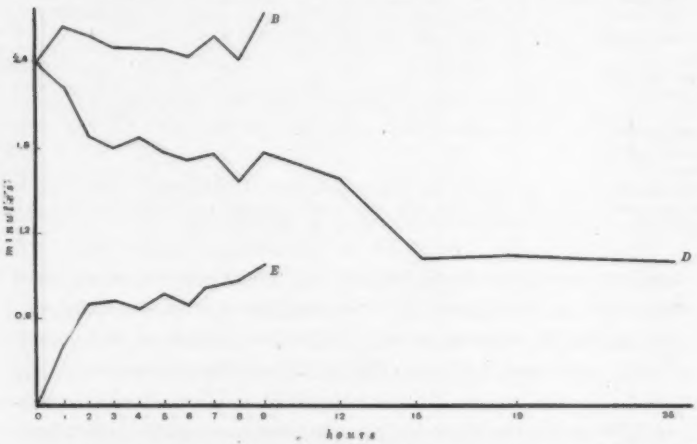


FIG. 3. *Paramecium.* Survival time of starved and yeast fed Paramecia in KCN. (See Table VI.) The abscissa represents time in hours between division and death of Paramecia. The ordinate represents survival time in minutes. Curve *B* represents the average survival time of yeast-fed Paramecia. Curve *D* represents the average survival time of the starved sisters of *B*. Curve *E* represents the difference between the average survival times of the fed and starved sisters. That is, it is the difference between the curves *B* and *D*.

death point. It is important to notice that the average difference in survival time of thirty pairs immediately after division, and the average difference after the sisters had been starving 25 hours, are exactly the same (viz., .02 minutes), even though the actual survival time of sisters immediately after division and after starving for 25 hours is very different. This close correspondence is probably due to the fact that in the case of *Para-*

*mecia* division is practically equal; that is, there is as much food in one daughter cell as in the other. While in the case of *Didinia* we often have unequal division, and accordingly the difference in survival time is greatest at the age of one half and one hour.

Attention may be called to the fact that here the *Paramecia* which were left to age in tap water and food (that is, yeast) were more resistant than those left in tap water alone, and that therefore the tap water as such had no deleterious effect, as might have been suspected in the case of *Paramecia* grown in hay infusion. (Cf. experiment II. above.)

## Discussion.

It is evident then from the preceding six experiments that young *Paramecia* and starved *Paramecia* are more susceptible to KCN than old (fed) *Paramecia*. In the case of *Didinia*, although as far as average differences are concerned, the reactions of the starved and fed are like that of *Paramecia*, when the survival times of the fed animals are considered alone, it is seen that there is a decided rhythm, the resistance increasing up to the fourth hour and then decreasing to the sixth hour, at which time a large percentage has divided. In view of this latter fact it is of interest to note the similar results of Lyon and others in regard to the rhythmic susceptibility of sea urchin eggs. The results of Lyon's experiments (Lyon, '02) on the susceptibility of sea urchin eggs to KCN solutions show roughly, although clearly, that the susceptibility to KCN reaches a maximum and a minimum at stated intervals. About ten to fifteen minutes after fertilization the egg is especially suspectible to KCN. Again, after the first cleavage and after the second come susceptible periods with a rise in resistance in between. He was working on the supposition that KCN inhibits oxidations, and so it would be possible to test this further by depriving the eggs of oxygen in some other way. This he did by substituting hydrogen for air in the water in which the eggs were to develop. There was a decided similarity between this experiment and the previous one. There was a gradual loss of resistance during the first ten to fifteen minutes after fertilization. Then the resistance increased. This experiment was carried no further than thirty

minutes, and so it is uncertain as to whether there was a second and a third susceptible period after the first and second cleavages. He also investigated the effect of heat and cold on developing sea urchin eggs (Lyon, '04) and found that immediately after the entrance of the sperm the egg is more susceptible to heat than a few minutes later. The resistance decreases, reaching a low point just before division, then increases, reaching a maximum right after division, decreasing again to a low limit just before the second division. Similar experiments were performed with cold but they were not carried beyond the first cleavage, so here no rhythm was demonstrated, but he considers the existence of such a rhythm probable. Lyon ('04) also studied the carbon dioxide production of the developing sea urchin egg. His experiments here were not strictly quantitative in character, but he found a slight increase in $CO_2$ production in the first ten to fifteen minute interval following fertilization. Decreased $CO_2$ production followed. The interval during which the eggs were actively dividing into the first two blastomeres was one of active $CO_2$ production. This was followed by a period of lessened production with a second rise at the time of the second cleavage. Spaulding ('04) also found decided rhythms in the resistance of the developing sea urchin egg to both ether and hydrochloric acid. Mathews ('06) made a study of living eggs of *Arbacia* and *Asterias* and examined sections of eggs preserved at definite intervals after fertilization. Comparing Lyon's work on *Arbacia* and the condition of the egg at the various intervals, as shown by the sections, he concludes that the period of greatest susceptibility is immediately before and during segmentation, and that just after segmentation there is a period of great resistance. He also endeavored to repeat Lyon's work on *Arbacia*, using *Asterias* eggs. The results were unsatisfactory so far as sharp and decisive periods of susceptibility were concerned, but they showed clearly that the eggs in certain stages were more susceptible to KCN than in others.

Moore ('15), in his work on artificial parthenogenesis, found that the greatest number of eggs, which had been previously treated with butyric acid, developed when treated with the hypertonic sea water at 40, 60, 90–100 and 115–125 minutes

after the fatty acid treatment. In the case of normally fertilized eggs where hypertonic sea water would not have the above "curative" effect, but could be nothing but injurious, he found that with an exposure of 40 minutes a maximum susceptibility occurred just after fertilization and immediately before and during each cytoplasmic division; while maximum resistance is 35 to 45 minutes after fertilization and just after each division. R. S. Lillie ('16) finds an osmotic swelling taking place in *Arbacia* eggs when placed in dilute sea water. The rate of this swelling depends upon the degree of dilution of the sea water and the condition of the eggs. The fertilized eggs have a higher resistance to the osmotic swelling, and consequent cytolysis than the unfertilized; but shortly before the appearance of the cleavage furrow the resistance rapidly declines to a minimum at the time of the appearance of the furrow. Immediately after the completion of the furrow the former resistance returns. A similar change is found at the second and third cleavages, and probably occurs at all cell divisions.

In looking for a cause or causes of the variability in resistance of *Didinium* and *Paramecium* to KCN we are reminded at once of the fact that Lyon found the periods of susceptibility to KCN of the sea urchin eggs to correspond to those for hydrogen which might lead to the conclusion that oxidations are interfered with. Warburg ('14) and Loeb and Wasteneys ('13) have found that the oxygen consumption of the sea urchin egg is actually decreased by the presence of KCN. Child ('15) finds that stimulated flat worms are more susceptible to KCN than the unstimulated, and also that the worms which he found to have a low resistance to KCN had a high rate of $CO_2$ production as determined by Tashiro's biometer. He concludes from this fact that there is a relation between the resistance to KCN and "the rate of metabolic reactions, or certain of them, probably the oxidations." He also finds that there is a marked difference between old and young *Planaria*, and between starved and fed *Planaria* of the same age. The young worms and the starved worms show a greater degree of susceptibility than the older worms and the well fed worms, respectively. The results given above for *Paramecium* and *Didinium* are the same as those found

by Child on *Planaria*. Now, considering the parallelism between resistance to KCN and $CO_2$ production and also the resistance to KCN of stimulated and unstimulated worms, Child concludes that the young worms have a greater metabolic rate than the old ones, the starved greater than the fed; and since my results on *Paramecia* with respect to survival time in KCN seem to be identical with these on *Planaria*, I was inclined to accept this conclusion and apply it to these protozoa. But recent work in this laboratory on the actual oxygen consumption of *Paramecia* indicates that in the case of this animal at least KCN has no effect on oxidations, even up to concentrations which kill in a few hours (Lund, '18). And so we must search for the explanation of these differences in some other direction.

If the permeability of the plasma membrane of the protozoan, like that of the surface of the sea urchin egg, changes at different times in its history and with different nutritive conditions, we might use that in explaining its various reactions to KCN. R. S. Lillie ('16) has shown that in the case of the egg, permeability is greatest at division and less between divisions; and, as stated above, according to Lyon and others, susceptibility to KCN is greatest at division and less during the time between divisions. *Didinia* show a similar behavior, the resistance increasing up to the fourth hour, but then decreasing again to the fifth hour when 37 per cent. of them have divided. The results for *Paramecia*, however, do not give a curve of this shape; although the resistance increases to a marked extent in hay infusion, and to a smaller extent when fed yeast, up to the very time of the beginning of the formation of the constriction. My data on the reactions beyond this point (that is, the time when the cell begins division) are insufficient to warrant conclusions. However, my impression from the few *Paramecia* which did happen to be in the beginning stages of division while in the KCN is that the resistance does not decrease previous to the time of separation.

If, on account of this parallelism with the results on the egg, it is conceded that the susceptibility of *Didinia* is due to an increase in permeability, the conclusion might be justified that the high susceptibility of the starved animals *is due to the same*

*thing*. If this is the case, it is obvious that there is an inconsistency in relating permeability and rate of oxidations; for the egg at division has an increase in permeability and also, if we may depend upon the limited data available on this point, an increase in oxidation rate, while a starved *Paramecium* or *Didinium* has, according to this hypothesis, an increase in permeability and, according to the facts, a *decrease* in rate of oxidation. The latter has been found in some recent work in this laboratory, which is as yet unpublished.

It is interesting to note, in regard to the lack of resistance to KCN of the starving cells, that this is in accord with the common experience that tissues suffering from lack of normal blood supply offer less resistance to various toxins and infections than tissues which are well supplied with blood; *i. e.*, well nourished.

It is evident that the experiments given above on *Paramecium* and *Didinium*, and the results on eggs of echinoderms by others, and those on lower invertebrates, offer a promising avenue of approach to an understanding of what some of the fundamental conditions in the cell are which determine the degree of resistance of cells to toxic substances.

## Summary.

1. An attempt was made to discover what factor or factors are responsible for the observed differences among individuals of a pure line of *Paramecia* and *Didinia* living in the same culture medium; *e. g.*, hay infusion. What are the differences in a protozoan cell which cause difference in response to apparently identical external conditions at different times?

2. Survival time (resistance) of *Paramecia* and *Didinia* in solutions of KCN was selected and used as an index because of the generally supposed relation of the toxic action of KCN to the rate of oxidation in cells.

3. All the data on survival time of *Paramecium* and *Didinium* were obtained by observations on individual cells and not by estimating the average survival time of large numbers of cells as is usual in such experiments. In this way the variation in resistance of the cell at different times during the period between cell divisions was followed accurately and is summarized in curves.

4. The cessation of movement of *Paramecium* is a less variable "end point" than cytolysis for determining the death point in KCN solutions.

5. *Paramecia* can be cultivated in pure line mass cultures in tap water when fed compressed yeast. In this way the chemical composition of the medium and food can be kept more constant than has been possible previously in work on *Paramecium*.

6. Resistance of *Paramecium* to KCN when allowed to feed on bacteria shows a marked increase, and when fed on yeast the resistance increases to a smaller degree from the time of division up to the following division. The resistance of *Didinia* to KCN when fed with *Paramecia* increased from the time of completion of division until a maximum was reached some time previous to the second division, and then gradually fell before the second division. This rhythm is directly comparable to that found by Lyon and others on echinoderm eggs.

7. When *Paramecium* and *Didinium* are prevented from obtaining food the resistance to KCN gradually decreases below its value at the completion of division.

8. Starvation of sister cells of *Didinia* results in a decrease in difference of survival time in KCN. This original difference in sisters is apparently due to the fact that the food content is not always distributed equally to the daughter cells at division. In *Paramecia* the distribution of food is practically equal, and here the average difference in survival time between sisters is the same immediately after division as it is after a period of twenty-four hours. This small observed difference is however within the limits of experimental error.

9. The difference in resistance of fed and starved *Paramecia* and *Didinia* and the rhythmic change in resistance in the case of *Didinia* between cell divisions which is closely similar to that found in echinoderm eggs can at present be best explained by assuming that it is due to change in permeability of the cell. Penetration of KCN into the cell and hence its toxic action as measured by survival time, is dependent upon the degree of permeability of the cell at different times. On this assumption rhythm in susceptibility depends primarily upon rhythm in permeability. The possible relation between rate of intracellular

oxidation or nutrition and cell permeability is at present in the absence of sufficient data an open question.

April 15, 1918.

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# BILATERAL TENDENCIES AND HABITS IN THE TWENTY-RAYED STARFISH PYCNOPODIA HELIENTHOIDES (STIMPSON).

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## INTRODUCTION.

The purpose of the investigation upon which this paper is based is to show to what extent the radial symmetry in *Pycnopodia helienthoides* is disturbed by its bilateral tendencies.

The nature of the investigation has been primarily based on observations on the animal in its native home, and as little as possible have conditions been introduced during experimentations which would deviate from that of its natural abode.

The carrying out of this experimental work on *Pycnopodia* was done in connection with other work at the University of Washington, during the year 1915, and during part of the summer of 1916. The experimental work was done at Bremerton, Wash., which is situated about twelve miles from Seattle. In some of

the bays at Bremerton, *Pycnopodia* had congregated in superabundance, offering a considerable inducement to perform experiments on them there, rather than anywhere else on the Puget Sound. And although much time was consumed in traveling between Seattle and Bremerton, the abundance of specimens at the latter place compensated fully for the time thus lost. Occasionally a number of specimens were brought from Bremerton and planted in Elliot Bay at West Seattle for the purpose of studying them there, but when the salinity was found to fluctuate more at the latter place than at the former, it was thought unwise to study them in the new place as under normal conditions and therefore, Bremerton was selected as the most logical place for collecting these data.

## ECOLOGY.

### DISTRIBUTION.

*Pycnopodia helienthoides* is found along the Pacific Coast from central California to southeastern Alaska. It inhabits the entire Laminarian Zone, *i. e.*, from low-water down to a depth of fifty fathoms, or even beyond that. Very seldom is it caught above low-water mark. When thus found, it is generally in pools where the water remains fresh till the tide returns; when found on piles under docks, after the tide has run out, it is because of the fact that *Pycnopodia* does not readily move backward or sidewise, but uses the side established as fore-end, always as anterior end. In places where the bottom is sandy and void of food and where nearby piles are covered with barnacles, *Pycnopodia* may be found, at low-tide, above the water-line. As was clearly demonstrated by some of the samples that were brought to West Seattle and placed under an old dock there, at low-tide a goodly number of them were found hanging on the dry piles. These piles were covered with barnacles and the bottom below was very sandy and depleted of all kinds of food. The writer has never found a duplication of this in other places, however. It may be that the star in this particular instance was exceptionally hungry and did not notice the decreased pressure as the tide receded; in places where food is readily obtained elsewhere, *Pycnopodia*, as a rule, keeps below water.

The Puget Sound region is well suited to *Pycnopodia*. The

temperature, moderate during the entire year, the many sheltered bays and the great abundance of food are undoubtedly contributing factors for the presence of this species of starfish in large numbers. Professor Kincaid, while dredging for marine specimens in connection with work at the Puget Sound Marine Station at Friday Harbor, has come to places where he has got the dredge absolutely full of *Pycnopodia*.

The coast-lines along the shores of the bays at Bremerton, especially along the shores of the town, are literally covered with starfish of various species. The most numerous is the *Evasterias troschelii* (Simpson); then, the species *Piaster ochraceus* (Brandt); *P. paucispinus* and *Dermasterias imbricata* are also found as well as *Pycnopodia*. The numeric ratio of the four former is one *P. ocraceus* to twenty-five *E. troschelii;* one *P. paucispinus* to fifty *E. Troschelii*, and as many *Dermasterias* as *P. ochraceus*. There is a considerable variation in the number of rays of *E. troschelii*. The normal number is of course five, but many have six rays, others seven, and still others four. These species occur in certain belts along the shore, *e. g.*, *Evasterias* occurs the farthest up; in the lower part of this belt, and extending below, is *P. paucispinus*. *P. ochraceus* is seldom found above low-water mark, and together with it are *Dermasterias* and *Pycnopodia*. This may then be taken as the upper limit for *Pycnopodia*, and the lower limit as that as stated before, about fifty fathoms. It is in this respect common with members of the related family Brisingidæ (Sars, 1875), *e. g.*, *Labidiaster radiosus* (Lütken) which, according to Ludwig, occurs in the south Atlantic and south Pacific oceans, in the littoral zone. Verrill, referring to the same species, says: "Unlike the other forms of Brisingidæ, it lives in shallow water as well as at considerable depths. It is found on both coasts of Patagonia and off Cape Horn, etc."

## Movements.

### *Righting Reactions.*

When experimenting on righting reactions care was taken so that the specimens would be under as normal conditions as possible, and no restrictions were put on them. A large number of trials were made in nature without taking any data; but the

striking similarity of reactions when disturbed gave cause for recording carefully the actual means and manners of righting. A small specimen from shallow water demonstrated very strikingly when put in a large tub of sea-water that *Pycnopodia* has a marked control of its radial muscles. That is to say, it has greater power of correlation of muscles than perhaps any other starfish. Out of fifty trials on the same specimen, forty-six turns were toward the same side, three turns toward the opposite side, and one turn at right angles. After one becomes familiar with the general method of righting reactions which *Pycnopodia* follows, one can easily notice any other method it may adopt or chance upon in an effort to right itself. It makes no difference as to the direction of the rays of light, the star uses the anterior end, as initiative end, in turning. To illustrate: When this species is turned on its back it immediately commences to put its anterior end under its back (Figs. 1, 2, Plate I.), attaching the sucker-feet to the bottom and pulling with them, while with he opposite end, also curved under, it pushes until the dorsal side is up. To right itself in the direction most usually followed, a unified impulse is apparent as soon as the star has been turned on its back. One may keep experimenting in this way almost indefinitely with the same result as above. The turning over toward the right angle happened perhaps accidentally, in that one ray which was at right angles to the anterior ray caught hold on the bottom first, and the pull begun in that direction, the other rays coöperating, pushing or pulling. Such a turn, however, as seen in the appended table, takes by far much longer time than even the slowest righting reaction toward the posterior or anterior ends. Therefore, when abnormal rightings occur, they are apparently due to fatigue or confusion, as the movements at the beginning of experiments are always in the same direction, provided that the specimens be taken from fresh and tolerably shallow water. Experiments on specimens which had been kept in a vessel for some time, invariably gave fluctuating results; they would act as if unbalanced, a fact undoubtedly due to the deoxygenation of the water. Other factors, external and internal enter in also. Externally there is the change in temperature as well as the depletion of oxygen; internally there is the effect of

external conditions. The lack of oxygen, or the insufficient amount of it causes, as demonstrated by Loeb (6) and others, ". . . at first molecular, and later morphological changes in the cells, which in turn are the cause of the supension of life-phenomenon." No apparent morphological changes were observed on the star from the lack of oxygen, but the unbalanced behavior seems to indicate some changes to have taken place. The effect of the stimuli received from the righting impulse, whether it be a normal or an abnormal individual, is to move away from the place of disturbance. (Fig. 3, Plate I).

Such definite movements tend to show that *Pycnopodia* has some established habits. In this respect it is indeed very much different from the common starfish, *e. g., Asterias forbesi* and *A. vulgaris*, which, according to Dr. Coe (2), "Do the same thing, under the same conditions, in a number of different ways, and never do the same thing twice in exactly the same way."

Jennings (4) also makes a statement similar to that of Dr. Coe, when he says: "The starfish (*Asterias firreri*) is not hampered by any consideration of anterior or posterior; it may move with any of its rays in the lead, or with any interradius in advance, or indeed in any intermediate direction, so that its possibilities as to direction of locomotion are really unlimited. In the same way, it may right itself in an indefinite number of different ways." *Pycnopodia*, however, has already formed certain definite modes of behavior. Part of these is indicated by its righting reactions. The speed of righting, as seen below, is not improved by repetition, but the method of righting indicates clearly that it possesses a more definite control of its muscles, or has a more definite method of righting itself than has the common starfish. In regard to habit formation the result is negative.

Jennings (4), in describing the righting reactions of the common starfish, says: "After repeated experiences by a given individual, there was no improvement in the performance of this reaction, either in the time taken, or in the movements employed in accomplishing the righting."

The first eleven trials show uniformity in speed of righting reaction; the first three show the same speed. Then there is a very rapid turn, taking only half the time of one of the previous

RATE OF RIGHTING REACTIONS: Experiments carried out on April 4th, 1915. Temperature 10° C.

| *Number of Trials.* | *Time.* | |
|---|---|---|
| 1st trial | 60 sec | The first 20 trials are in the same direction, *e. g.*, toward the anterior end. The average speed of righting in these trials equals 66 sec. |
| 2d " | 60 " | |
| 3d " | 60 " | |
| 4th " | 30 " | |
| 5th " | 50 " | |
| 6th " | 60 " | |
| 7th " | 40 " | |
| 8th " | 40 " | |
| 9th " | 40 " | |
| 10th " | 60 " | |
| 11th " | 70 " | |
| 12th " | 70 " | |
| 13th " | 70 " | |
| 14th " | 65 " | |
| 15th " | 70 " | |
| 16th " | 65 " | |
| 17th " | 75 " | |
| 18th " | 85 " | |
| 19th " | 110 " | |
| 20th " | 120 " | |
| 21st " | 70 " | Righting movements toward posterior end. |
| 22d " | 50 " | Righting movements toward the anterior end. The average speed equals 55 sec. |
| 23d " | 50 " | |
| 24th " | 55 " | |
| 25th " | 60 " | |
| 26th " | 60 " | |
| 27th " | 50 " | |
| 28th " | 40 " | |
| 29th " | 40 " | |
| 30th " | 60 " | |
| 31st " | 90 " | |
| 32d " | 70 " | Righting movement toward posterior end. |
| 33d " | 40 " | Righting movements toward the anterior end. The average speed equals 52.7 sec. |
| 34th " | 30 " | |
| 35th " | 30 " | |
| 36th " | 40 " | |
| 37th " | 40 " | |
| 38th " | 44 " | |
| 39th " | 50 " | |
| 40th " | 55 " | |
| 41st " | 60 " | |
| 42d " | 60 " | |
| 43d " | 65 " | |
| 44th " | 65 " | |
| 45th " | 70 " | |
| 46th " | 90 " | |

47th " ..... 80 " } Righting movement toward the posterior end.

48th " .....180 " } Abnormal turn toward right angle of anterio-posterio ends.

49th " ..... 50 " } Righting movements toward anterior end.

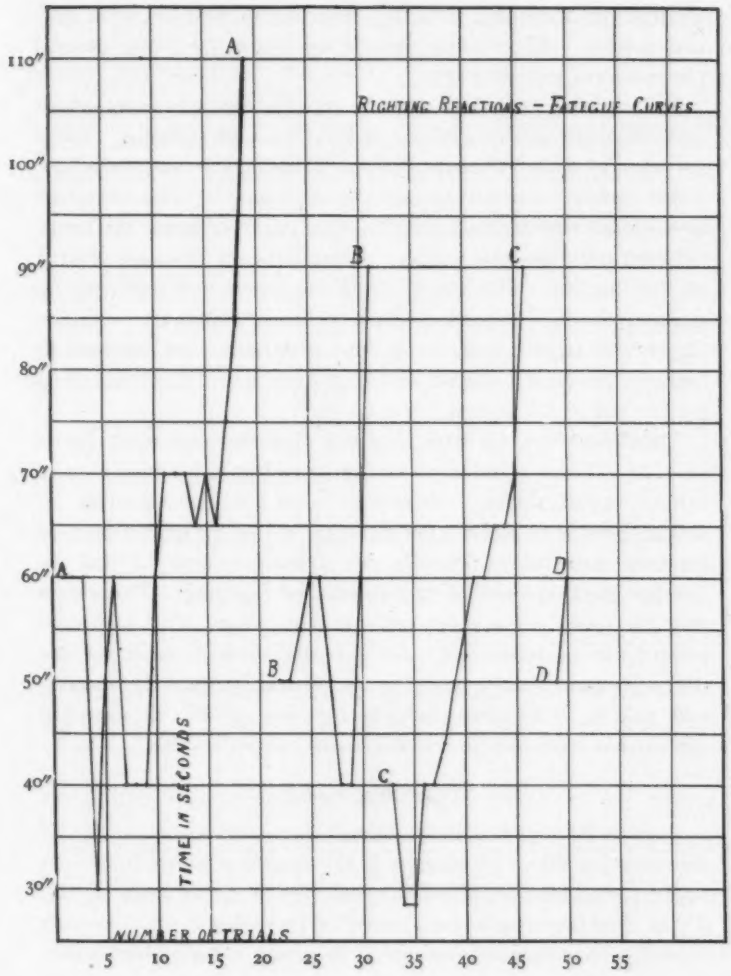
50th " ..... 60 " } The average speed equals 55 sec.

The total average speed of the normal turns equals 57.1 sec.

trials. At this point the writer thought that the star was learning to do the righting more quickly than at first, but the subsequent trials disprove it. After this last trial, three of equal speed follow, each taking only 40 sec., but after these there is a slowing up in speed, an indication of fatigue. The speed is now gradually diminished until at the twentieth trial, when it takes two minutes in righting itself. Following this is a righting toward the opposite side, at a speed of 70 sec., but the succeeding ten trials are all again in the same direction as at first, and with an average speed of 55 sec., per righting. Then follows another turn toward the posterior end, with a speed of 70 sec. This is followed by fourteen turns toward the anterior end with an average speed of 52.7 sec. Now follows the abnormal turn described above, a turn toward the right side, with a speed of three minutes, followed by two turns toward the anterior end with an average speed of 55 sec. per righting. Here ends the experimenting on right movements on this particular individual. The total average speed per normal rightings is 57.1 sec. per righting. The series of rightings in one direction indicate fatigue toward the last, or a decrease in speed of righting; a turn toward the posterior end is followed by an increased speed in the succeeding rightings toward the anterior end, which, however, soon decreases. Whatever this may indicate, one thing is apparent: that *Pycnopodia* is able to right itself in more than one way, but that it turns more easily and far more frequently toward the anterior than toward the posterior end; that it never turns twice in succession toward the posterior end.

These trials on righting reactions were made on a small individual of four inches in diameter, and which had 13 rays. This specimen was kept in a cage of six by four by one feet in size. The cage consisted of a wire-aquarium which was anchored to a

FIG. 1.


RIGHTING REACTIONS – FATIGUE CURVES
110″
100″
90″
80″
70″
60″
50″
40″
30″
A
B
C
D
TIME IN SECONDS
NUMBER OF TRIALS
5
10
15
20
25
30
35
40
45
50
55

float. The specimen was fed on small shell-fish of the families Cardiidæ and Leptonidæ; on gasteropods: *Amphissa versicolor* and also on crabs (*Pugettia gracilis, Cancer orgonensis*). Subsequent experimenting with this specimen showed the same general results. When using mature specimens the same general phenomenon was observed.

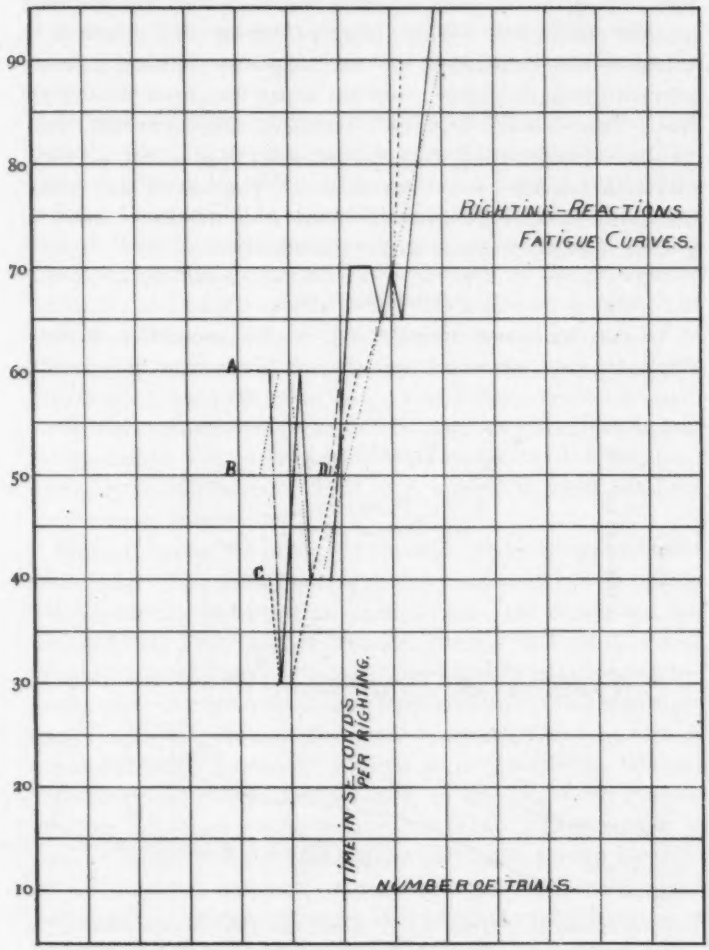
The righting reactions may be represented by curves which may show the whole working of fifty trials of righting. These curves also show the indication of fatigue, and are, therefore, called righting reaction-fatigue curves (Fig. 1). The abnormal or confused rightings are omitted from curves, hence the break between the respective curves. The number of trials are plotted on the abscissa. The speed in righting reaction is indicated by squares on the ordinates which in turn stand for minutes. Curve *AA* equals trials 1–19 (the twentieth trial omitted in figure); curve *BB* equals trials 22–31; curve *CC* equals trials 33–46; curve *DD* equals trials 49–50.

These curves when superimposed show an interesting figure (Fig. 2). There is the same picture as in Fig. 1, an indication of fatigue, toward the last. However, curve *AA* has an average reaction speed of 66 seconds per righting; curve *BB* has an average reaction speed of 55 seconds per righting; curve *CC* has an average reaction speed of 52.7 seconds per righting. This shows that the speed per average reaction is increased after each turn toward the posterior end, and that may indeed count for the difference in increase of speed in the turnings toward the anterior end, that is, it becomes easier to turn toward the anterior end after it has tried to right itself in some other direction.

### *Tidal Movements.*

*Pycnopodia* moves rapidly enough so as not to be caught by the outgoing tide. Its success in this regard is partly due to its habit, partly because of its physical need to be submerged, and it has therefore developed a speed of movements great enough to enable it to keep submerged all the time. During high-water it may move a considerable distance above low-water mark, and during such time be found on grounds which will be dry at low-water. In such cases it will move a little ahead of the tide,

FIG. 2.

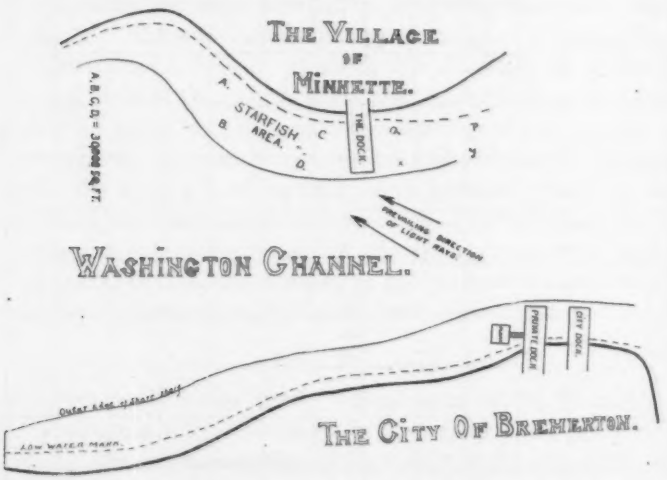

90
80
70
60
50
40
30
20
10
RIGHTING-REACTIONS.
FATIGUE CURVES.
A
B
C
D
TIME IN SECONDS PER RIGHTING.
NUMBER OF TRIALS.

as the tide runs out. The rate of speed of such distance-movements depends on the circumstances. For example, when instigated to move by some artificial means, as by being turned on its back, brought from deep water to shallow water, the rate was 2.5 feet per minute. When, however, during tidal recession it would remain stationary on its temporary feeding ground, selected during high-tide, until the water measured about 2.75 feet. Then it would begin to move down to deeper water, continuing moving until it had reached a depth of about 4.5 feet, and at a rate of 3.5 feet per minute. This shows that when instigated to move by a natural stimulation its rate of speed is greater than when caused to move artificially.

*Irritability Limit.*

Another indication brought out in this connection is that *Pycnopodia* is sufficiently sensitive to pressure so as to start

FIG. 3.




moving down to deeper water when the tide has receded to a certain level. As a matter of fact, out of seventy-five specimens that were examined during one low-tide on a given area (Fig. 3), only two were at the shallow depth of 2.5 feet. Specimens brought from deeper water to shallower water, that is from any

depth (see topic on distribution, p. 233) to a depth of about three feet, will move down to a depth of at least five feet. Then the irritability limit ranges between the pressure of 2.5 feet and 5 feet of sea water. The average depth of the 75 specimens referred to above, on an area of about 30,000 square feet, was 4.76 feet. The minimum pressure in this irritability limit, to which *Pycnopodia* is adapted, is seen to be about 1.13 lb. to the square inch.

An irritability limit may seem improbable when it is considered that this starfish has been found on piles above water and on dry tide-flats during low-tide. Either of these cases is very extraordinary, in that tidal recession on tide-flats is sometimes great enough even to catch swiftly swimming fishes. On piles onto which the gluttonous starfish has been lured by the presence of barnacles, the tidal recession is indeed the slowest possible, but in this case the star is in a very awkward position to move. It has already been stated that the righting movements are almost invariably toward the same side. This is also the case relative to distance-movements. One end is, much more so, than in any other starfish, the anterior end; *Pycnopodia* has a decided bilaterality in this regard. That is, it is used to move with the same side as anterior end.

When it moves the rays are curved at the ends, the eye-spots wide open. The second pair of primary posterior rays is pulling along with the odd posterior primary ray; the sucker-feet are engaged in a lively and continuous march. The third pair of primary posterior rays is occasionally lifted foreward spontaneously, while the first pair of the post-larvel rays which is at right angle to the anterio-posterior rays, when the star is at rest, is lagging somewhat behind. During such movements, this anterior end is decidedly used as such, so that when the star encounters obstacles which it may not crawl under or over, it merely, as an ordinary bilateral animal, turns toward its right or left side. It does not, in such instances, utilize its radial symmetry by starting to move backward or to the right angle or to any other angle to its longitudinal axis.

*Migratory Movements.*

Statements have already been made to the point that *Pycnopodia* possesses locomotive powers great enough so as not to be caught by the outgoing tide, provided that the land is not too flat. The rate of tidal recession, even during times of extreme low-tide, will not be faster on a slope of six inches to the distance of twelve and a half feet, than *Pycnopodia* is able to keep pace with. In addition to these kinds of movements which have been mentioned, *Pycnopodia* seems to move according to seasons of the year. Seasonal movements, as they may be called, have three underlying primary causes, to wit: reaction toward light, search for food, and the breeding impulse. These three are causes for extensive migrations.

*Reaction toward Light.*—During the summer time *Pycnopodia* is found, as a rule, in deeper waters. If found in shallow water during this time of the year it will almost always be in shaded places, as under docks, under kelp, algæ or on the shaded side of a bay. This seems to point to negative heliotropism. In the spring, 1915, during the breeding season, *Pycnopodia* was found actually congregated in large numbers on the sunny side of the bay, but two months later there was not a single specimen on that side of the bay, while on the shaded side of the same bay a few were found under a dock, but farther out than during the spring and winter. Movements of this nature vary, however. As was seen during the summer of 1916, when the whole summer was exceptionally rainy, cloudy and cool, *Pycnopodia* seemed to take advantage of such conditions and remained in shallow water all the time. August, however, proved to be more sunny and warm than the early part of the summer, with the effect that the rays of the sun became annoying to the star. This unexpected and intense light caused every single specimen, as far as could be observed, to move away, either down to deeper water or to hide under some object. The dock, as an illustration (Fig. 3, Minette side of the bay, starfish area *ACBD*), served as a satisfactory shading place, where *Pycnopodia* actually congregated in a continuous layer from three feet depth to as far as could be seen at low-water. The question arises: How did the star find the dock to hide under when the sunny days of August suddenly came?

The dock, as seen in Fig. 3, is located near the point of the Minette side of Washington Channel, opposite the City of Bremerton. On no occasion has the writer observed *Pycnopodia* on the outside of the dock or in the area *QPXY*, but always on the inner side, toward the Washington Channel. The sudden continuous sunshine which came in August with the temperature the highest in the year soon made itself felt to the negative heliotropic star. Observation on the direction of the rays of light during the middle of the day explains this phenomenon. The light rays of the late forenoon and early afternoon are up the channel, or diagonally from the dock toward the starfish field, *ACBD*. The cause for the movements and orientation of the star in this particular case must be sought in the phenomenon of the effect of the direction of the most intense rays of light.

Loeb (6), working on *Spirographis spallanzanii*, found that rays of light, if sufficiently intense, are unequivocally able to determine the orientation. In summarizing the causes for moving to or from the source of light, he says: "The direction of the median plane of an animal coincides with the direction of the rays of light. Light of a constant intensity acts as a constant stimulus, in animals as well as in plants." These statements seem to explain why *Pycnopodia* "found" the dock. The star, however, moved toward the source of light, and according to Loeb's conception of the behavior of heliotropic animals, that should make *Pycnopodia* positively heliotropic, for, "Positively heliotropic animals will move toward the source of light, even if in so doing they go from places of greater intensity of light to places of less intensity." When the star, however, came under a good shading object, such as the dock offered, it remained there for several weeks, and did not venture away from the dock in either direction.

As was said above, *Pycnopodia* occurs in shallow water during the spring and late winter, and in deep water during the lightest part of the year. The early part of summer and late part of winter are the regular migratory periods. The causes for migratory impulses have been designated as three: the effect of the rays of light, search for food and the breeding impulse. But in a rather large number of cases movements of *Pycnopodia* at the

two stated periods may not be effected by any one of these causes, separately. For still other factors enter it, and, therefore, more frequently several causes may coöperate, resulting in definite movements at the same time. Thus the variable conditions within the animal may cause different reactions toward light at various times.

Loeb (6) has shown that the caterpillars of *Porthesia chrysorrhœa*, after fasting through the winter, are energetically positively heliotropic; but that after these animals have eaten, heliotropism is not shown so definitely Plant lice become positively heliotropic only after they have fed; the larvæ *Musea vomitoria* are energetically negatively heliotropic only when fully grown, etc.

Heliotropism in *Pycnopodia* is, however, only a partial cause for migration or distance-movements, and the periods of migration are not very definitely marked; they are quite extended in time. Various external conditions, as well as internal, contribute to this.

*Search for Food.*—The food problem is another factor which determines the distance-movements in *Pycnopodia*. It will, like other starfish, move according to the distribution of food. If *Pycnopodia* finds its surroundings scarce in food material, it has no difficulty in taking leave for different grounds. In fact, as it has been found in bays at a depth of twenty fathoms where there would be a great variety of food, *e. g.*, sea-urchins (*Strongylocentrotus dröbaciencis*), *Pectens*, *Yoldia*, etc., when conditions for it would not be favorable to remain on other grounds, such as in shallow water during the summer, indications seem to point that way.

*Breeding Impulse.*—Temperature together with the impulse to breed are still other causes for migration. The temperature below ten fathoms is comparatively constant during the entire year, while the temperature at the surface varies quite considerable during the year. The lowest and highest temperatures during the year 1915 were 9° C. and 16° C. respectively. That *Pycnopodia* should move up to shallow water during the spawning season seems reasonable, for the temperature in deeper waters is undoubtedly lower than at the surface even during the spring. Reference was made

above to the fact that *Pycnopodia* was found in large numbers on a certain side of a bay, during spring, while it was totally absent from these grounds later on, during the summer. As a matter of fact, during the spring of 1915, on the area *ACBD* (Fig. 3) more than one hundred specimens were counted, while in July of the same year not a single specimen was seen on the same area. The change in habitat was perhaps due to the intense sunlight, as during the cool and rainy summer of 1916, a large number was seen on the same area until constant sunny weather ensued. As demonstrated above, this is an indication of positive heliotropism. The habitat of *Pycnopodia* ranges over the entire belt of the laminarian *Benthos;* it lives as a rule in deeper water during the warm and light part of the year, and in shallower water during the spawning season and during the darker and colder part of the year.

## FEEDING.

### *Kinds of Food.*

The feeding habit of *Pycnopodia* is striking and very interesting indeed. It lives on such types of mollusks as clams and gasterpods; on crustaceans, such as crabs and barnacles; on other echinoderms, such as common starfish and sea-urchins; on porifera, and on algæ.

### *Methods of Feeding.*

The methods of feeding are by engulfing and sucking. Large rocks up to a pound in weight have been found in the stomach. These were engulfed when sucking on barnacles, because markings of barnacles could be seen on the rocks. The large horse clam (*Schizothærus nauttalli*) cannot be engulfed on account of its enormous size. But when *Pycnopodia* encounters *Schizothærus*, it rolls itself around the latter and proceeds to digest it. On one occasion the writer found the star in the act of consuming one of these large horse clams. The extraordinary large syphon was forced into the mouth of the starfish while the everted stomach was folded around the syphon. The whole star had in fact, rolled itself around the clam, endeavoring to pull it open. This pulling on the part of the star had, however, no effect on the clam, for, although the latter might have relaxed to the continuous pull of the sucker-feet, it was of no avail because the folds

of the stomach of the star could not reach the vital parts of the clam exposed by the relaxation of the adductor muscles. The reason for the failure to kill the clam was due to the fact that the syphon of the clam, as stated before, was extended into the mouth of the star. The star seemed to have no effect on the clam, except that the syphon was partly digested around the edges of its apertures. Otherwise the clam seemed to have enjoyed the gastric juices of the star; it was still alive and behaved like unmolested clams of the same kind, when it was freed from its pursuer. Small clams are more easily preyed upon. Ordinary cockles are as a rule engulfed. In opening cockles or smaller clams, *Pycnopodia* holds its prey until it has a chance to force some of its everted stomach into the shell-fish, and having succeeded partly in this respect, it digests its prey piecemeal. In other instances, members of the family Natidæ (*e. g.*, *Nassa mendica*) were found in the stomach of this starfish, together with egg-bodies of Natidæ. As a matter of fact, *Pycnopodia* feeds especially on gasteropods, engulfing the smaller ones, as in the case of the last referred to. *Polynices*, though a monster of a slug, is a very easy prey, indeed, to *Pycnopodia*. In fact few, of this slug are found on grounds common to *Pycnopodia*. From experiment it was found that *Polynices* is very sensitive to the surface of this star. When keeping several *Polynices* in the aquarium with one *Pycnopodia*, it was found that the latter devoured three large (foot, about 8 inches long, 5 inches wide, and 4 inches deep) specimens in three days, leaving the shells on the outside. The method of feeding on these slugs is simply by suffocation. The slug seems to be incapable of living very long within its shell, and unless it is allowed to take in fresh water, when relaxing, it soon has to come out as a mere powerless, defenseless matter of flesh. When, therefore, *Pycnopodia* has *Polynices* in its control, the latter must very shortly give in to the former. In still other instances, remnants of crabs were found in the stomach of this star also. In this connection, experiments on feeding it with crabs were actually performed. Having stabbed a crab (*Magister productus*), it was thrown into the water so that it would fall down beside or on the top of the star. In one such instance, the latter seized the crab-fragments

with its pedicellariæ and crawled away with it to deeper water, and then started to feed on it there. In another instance, the star started to feed on the dead crab at once. In so doing it put the rays over the crab, tucking it under itself, rising on the tips of its rays and in this way proceeded to devour its meal. In yet another instance it was found that *Pycnopodia* was feeding on other starfish (*Evasterias troscehlii*), and this even though there seemed to be plenty of gasteropods and other mollusks present. It was also found to be feeding on *Strongylocentrotus dröbachiensis*. Finally *Pycnopodia* feeds on porifera and on plants—of the latter, kelps and algæ. This last kind of food is probably resorted to when clams and gasteropods are beyond its reach. Because of these various types of food that this starfish feeds on, it may well be said that *Pycnopodia* is omnivorous in its habits of feeding.

## Conclusion on Movements in General.

The fact that *Pycnopodia* always moves along one and the same axis, with the same side always as anterior end; the fact that it has a definite method of righting itself, as well as apparent voluntary behavior during feeding, seems to point to dominating bilateral symmetry rather than radial symmetry. With such evidence of dominating bilateral symmetry, one should expect a nervous system more complex than that of the ordinary starfish. Indeed, something relative to experiments on the regenerative powers of this genus seems to point that way. The regenerative powers in *Pycnopodia* seems to be less than in the common starfish.

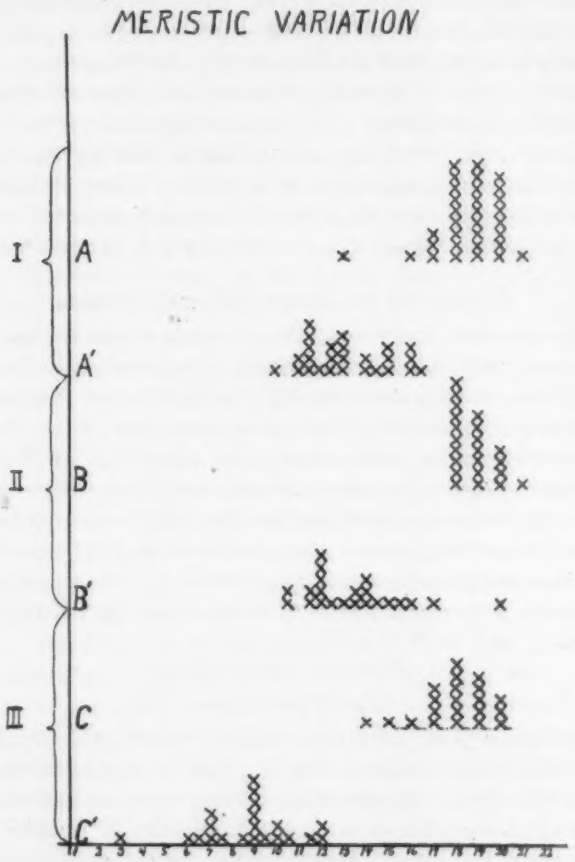
## Meristic Variation.

### *The Modal Curve.*

The modal point of rays in males, females and immature specimens is represented in Fig. 4. That of the female, *IIBB'* shows an abrupt demarcation, in that the average modal point of number of rays is reached before maturity, while that of the male fluctuates. This fluctuation, however, is the same after the modal point is reached, but is of course greatest, regardless of sex, among the immature, although that particular number which was examined showed a modal point much the same as that of the mature ones (Fig. 4, *IAA'*, males; *IIICC'*,

immatures). This was no doubt due to the fact that only a few small ones were found, and those found were approaching maturity. Curves representing the diameter may also be plotted (Fig. 4, *A'B'C'*). In each case the diameter which is measured

FIG. 4.




in inches from tip to tip of rays along the median axis is plotted on the abscissas, the number of specimens on the ordinates.

Ritter and Crocker (9) states that out of one hundred specimens that they studied not one was of the 18-rayed stage. Here, how-

ever, the modal point in both the male and female is seen to be 18. The adult cannot be determined by the number of rays, because the immature star may reach the modal point in number of rays, and like mature specimens go beyond the modal point, and even not reach maturity, while on the other hand mature male specimens may be found to have only 13 rays, which is reaching down into the immaturity limit. Mature specimens spoken of here have special reference to sexual maturity for that season. The modal point of both males and females is practically the same.

This is demonstrated by a number of specimens examined in the same region on the same day, and represented by Fig. 4, *I*, *A* (male), *II*, *B* (female). As a matter of fact, the average number of rays in the male, female and immature is almost the same, which goes to show that radial development occurs in the comparatively early part of the life of the individual star. On this occasion, the depth for and the diameter of every specimen was measured; and the number of rays present, and the sex was determined. These findings were as follows:

1. The average depth at low-tide for 75 specimens found in a belt 30 feet wide by $\frac{1}{6}$ of a mile long was 4.76 feet.

2. The diameter measured along the median axis from tip to tip of odd anterior and odd posterior rays for immature was 8.21 inches, and the average number of rays was 17.85.

3. The average diameter of the males was 13.5 inches, and the average number of rays was 18.56.

4. The average diameter of the female was 13.6 inches; the average number of rays was 18.8.

## SUMMARY.

1. *Pycnopodia* is highly bilateral when moving about in its natural abode. It uses the same side always as anterior end.

2. During righting reactions, this same side almost always takes the initiative and the greatest number of rightings are made in that direction. Repeated rightings do not improve its speed.

3. It is hardly ever found on dry land; its movements are swift enough so that it keeps below the tidal mark; it moves swiftest when stimulated from within.

4. In this, it seems to be partly governed by its sense to the pressure of minimum depths.

5. Three prime causes effect its migratory habits, to wit: (1) sensitivity to light of a certain intensity; (2) search for food; and (3) the breeding impulse. During the breeding season it is neither negative nor positive heliotropic; for it moves up from deeper water to shallower water independently of the directions of the rays of light. After spawning, however, it seems to be positive heliotropic.

6. It is omnivorous; it is carnivorous. It feeds mainly on Gasteropoda, but also on Pelecypoda, other species of starfish, sea-urchins, crabs, sponges, kelps and algæ.

7. The female seems to have reached a more definite number of rays before maturity, than has the male (see Fig. 4).

8. Maturity is not entirely dependent on the size of the animal; *i.e.*, the diameter varies: In immature up to 12 inches; in males from 10–16½ inches; in females from 10½–20 inches. The females are on the average larger than are the males.

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EXPLANATION TO PLATE I.

FIGS. 1–3. Photos of *Pycnopodia helienthoides* (Stimpson), showing righting movements; Fig. 1 shows the ventral side, and the beginning of righting. Note the outstretching tube-feet; Fig. 2 shows the same specimen ten seconds later, almost semi-righted: Fig. 3 shows the righted specimen, just after righting active y moving along. $\frac{1}{4}$ natural size.

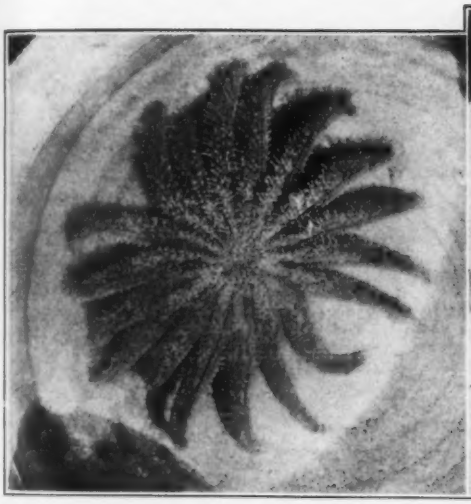

FIG. 1.

FIG. 2.

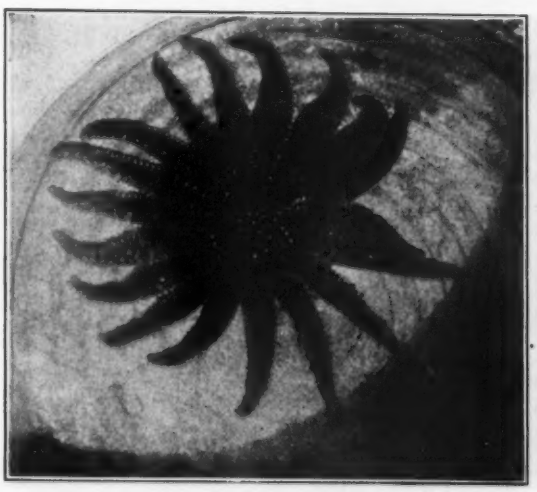

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FIG. 3.